

**35 U.S.C. § 112 Rejections**

Claims 1, 3-4, 13, 15-17, 19-29 have been rejected under 35 U.S.C. § 112, first paragraph, for an alleged lack of enablement for the full scope of the claims.

Applicants respectfully submit that amendments, which changed the recitation of “cell line” to “neuronal cell line”, obviate this rejection.

**35 U.S.C. S 103 Rejections:****Noble et al., Stocklin et al., Moses, in view of Reebe et al. and Yazdanbakhsh in view of Leder and further in view of Hammer**

Claims 1, 3-4, 6-9, 13, 15-29 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Noble et al., Stocklin et al., Moses, in view of Reebe et al. and Yazdanbakhsh in view of Leder and further in view of Hammer.

The Examiner asserts that Noble teaches transgenic animals and cell lines from any cell type of the animal body wherein the cell line comprises SV40tsA58 immortalizing gene and that Stocklin teaches a transgenic mouse wherein the human c-erb-2 is operably linked to the MMTV enhancer/promoter sequences such that the transgene is expressed in a variety of cells, and that Moses teaches a transgenic mouse expressing the huTGF- $\alpha$  gene under the control of an MMTV enhancer/promoter.

The Examiner admits that none of these references teach the use of the human neurofilament (NF-L) promoter to drive the expression of SV40tsA58, c-erbB-2 and TGF- $\alpha$  genes, but contends that Yazdanbakhsh teaches the human neurofilament NF-L promoter which regulates neuronal-specific expression. (OA p5).

The Examiner further asserts that Leder teaches a method of providing a cell line from a transgenic mouse encoding a transforming oncogene operably linked to mammary specific promoter MMTVLTR and use of transgenic mice for testing suspected carcinogens and protective materials. The Examiner admits that the combination of Noble, Stocklin, Moses, Yazdanbakhsh, and Leder do not teach the making of a transgenic rat encoding the same, but contends that it would have been obvious to have substituted the transgenic mice as taught by Noble, Stocklin, Moses and Yazdanbakhsh with a transgenic rat as taught by Hammer. The Examiner further contends that it would have been further obvious to test a material suspected of being a carcinogen with a transgenic rat as taught by Leder. Allegedly, one would have been motivated to do this because rats are widely used in biomedical research.

Applicants respectfully disagree with the Examiner's rejection and submit that the claimed invention is not rendered obvious by the cited art using the objective standard for obviousness under 35 U.S.C. § 103(a). A finding of obviousness under §103 requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v Deere*, 383 U.S. 1, 148 U.S.P.Q. 459 (1966). To establish a *prima facie* case of obviousness, all of the claim limitations must be taught or suggested by the prior art. M.P.E.P. § 2143.03.

Thus, the relevant inquiry is whether the prior art suggests each of the claim limitations of the invention, whether the prior art provides a motivation to combine the references, and whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. *See In re O'Farrell*, 853 F.2d 894, 903, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988).

In the present instance, the relevant inquiry is whether the Noble reference, when taken with Stocklin, Moses, Yazdanbakhsh, Leder, and Hammer suggests the claimed invention and whether the cited references themselves provide one of ordinary skill in the art with the motivation to combine them and a reasonable expectation of success of generating a neuronal cell line obtained from a transgenic rat comprising a conditional oncogene, transforming gene, immortalizing gene, or a cell cycle gene operably linked to a cell type specific promoter.

Applicants assert that the cited references neither suggest the claimed subject matter of the invention, nor do they provide a motivation to combine the references. Moreover, the references do not provide any expectation of success in achieving the claimed invention.

Noble appears to be directed to the use of a promoter that can be regulated by an exogenous agent. Such a promoter is referred to as a "non-constitutive" promoter. The teaching of Noble is directed at constructs and transgenic animals in which immortalization of cell lines can be easily controlled by external factors. Broad

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specificity promoters that are controlled by specific external signals are preferred as they allow cell lines to be derived for a wide variety of tissues.

In contrast, the object of the present invention is to provide a construct which preserves the differentiation specific features of particular cell type, in particular neuronal cells. There are few alternatives available that can provide large quantities of differentiated neuronal tissue *in vitro* for experimental work. The NF-L promoter disclosed in the present invention does not fall under the definition of the “non-constitutive” promoter of Noble in that it is necessarily expressed in neuronal cell lines to product the differentiated phenotype and is not activated by exogenous signals. Thus, the use of the NF-L promoter teaches away from the disclosure of Noble despite being coupled to a conditional immortalizing gene, since the promoter can maintain the desired features of differentiation in cell lines produced.

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Similarly, neither Moses nor Stocklin teach a cell type specific promoter. As stated in Stocklin p.201, “[d]espite the implication in its name, the MMTV LTR is not exclusively active in mammary gland cells. High level of expression have been detected in mammary epithelium, but expression was also found in the epithelial cells of salivary glands, lung, kidney, seminal vesicles, testes, and also in lymphoid cells in spleen and thymus.” (Emphasis added). Further, the expression in Stocklin et al. is not tissue specific since expression of the transgene was observed in the kidney, lung, mammary gland, salivary gland, and in the epithelial cells of the male reproductive tract.

Yazdanbakhsh does not cure the defect. It was not obvious when the applicants' NF-L construct was made that it would be expressed in precursor cells to product sufficient large T-Antigen to immortalize cells at the permissive temperature of the tsA58-SV40 gene because the NF-L promoter was thought only to be active in postmitotic neurons. (*See* Reeben et al., Biochem. Biophys Res Commu. Vol:192, pp.465-70, Tissue-Specific Expression of Rat Light Neurofilament Promoter-Driven Reporter Gene in Transgenic Mice," 1993). Expression from this promoter has not previously been observed in either embryonic stem cells or neuronal precursors. Thus, prior to the instant invention, it was believed that for an NF-L promoter to immortalize cells would either require de-differentiation of the cells reverting to a state similar to a stem cell, or that there is a population of neuronal stem cells that are isolated by the methods of this invention.

The Examiner has cited no evidence that anyone has produced a transgenic animal with a neuronal cell specific promoter operably linked to a conditional oncogene, transforming gene, immortalizing gene, or a cell cycle affecting gene.

In addition, none of the prior art references teach or suggest a neuronal cell line derived from a rat comprising a conditional oncogene, transforming gene, immortalizing gene or cell cycle affecting gene operably linked to a cell type specific promoter, particularly a human NF-L promoter.

It is an unexpected result that the 6kbp fragment of the human NF-L promoter, when introduced into transgenic rats, as opposed to mice, would result in a

completely unique tissue specific expression for the brain in the resultant transgenic rat. As explained in Reeben et al. ("Tissue-Specific Expression of Rat Light Neurofilament Promoter-Driven Reporter Gene in Transgenic Mice" Biochem. & Biophysical Research Commun. vol: 192, pp. 465-70), "[p]reviously, several groups have studied NF-L promoter driven reporter genes or transient expression of complete NF-L genes in different cell lines, particularly in rat pheochromocytoma PC12 cells, C6 glioma cells, → and fibroblasts. No cell-type specific expression was observed." Reeben at page 465 (emphasis added). This language suggests that an NF-L promoter would not be useful for specific immortalization purposes using rat cells. Therefore, it is an unexpected result that the 6kbp fragment of the human NF-L promoter, when introduced into transgenic rats, as opposed to mice, would result in a completely unique tissue specific expression for the brain in the resultant transgenic rat. See Application, Table 2, page 28.

The fact that rats are widely used in biomedical research does not provide the requisite motivation to generate a neuronal cell line from a rat having a human NF-L promoter.

In addition, the Examiner has not established a reasonable expectation of success. As stated in M.P.E.P. § 2143.02, the prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a reasonable expectation of success. The Examiner has not provided evidence that one of ordinary skill in the art, using the teachings Noble, Stocklin, Moses, Yazdanbakhsh, Leder, and Hammer would be expected to be able to produce the claimed invention.

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Respectfully submitted,

A handwritten signature in black ink, appearing to read "Ronald B. Hildreth", written over a horizontal line.

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**CHANGES TO THE PENDING CLAIMS:**

1. (Twice Amended) A neuronal cell line obtained from a transgenic rat, the cells of which comprise: [comprising:]
  - (i) a conditional oncogene, transforming gene or immortalizing gene or a cell cycle affecting gene operably linked to
  - (ii) a cell type specific promoter.
4. (Twice Amended) [A] The cell line [as claimed in claim 3] of claim 1 in which the cell type specific promoter is a human NF-L gene promoter.
6. (Twice Amended) [A] The cell line [as claimed in any of claims 1, 3,] of claim 1 or 4 in which the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene.
7. (Twice Amended) [A] The cell line [as claimed in any of claims 1, 3,] of claim 1 or 4 in which the conditional oncogene, transforming gene, immortalising gene or the cell cycle affecting gene is a C Erb  $\beta$ 2 gene or a TGF  $\alpha$  gene.
19. (Twice Amended) A method of testing a material suspected of being a carcinogen, said method comprising subjecting a rat according to claim 17 or 18 [or a rat produced according to the method of any of claims 13 or 15 or an ancestor thereof or cells or tissue from a cell line of any of claims 1, 3, 4, 6, 7, 8, or 9,] to said material and detecting neoplasms as an indication of carcinogenicity.
20. (Twice Amended) A method of testing a material suspected of conferring protection against the development of neoplasms, said method comprising



administering said material to a rat according to claim 17 or 18 [or a rat produced according to a method of claims 13 or 15 or an ancestor thereof or cells or tissues from a cell line of any of claims 1, 3, 4, 6, 7, 8, or 9,] and detecting a reduced incidence of development or neoplasms, compared to an untreated rat, as an indication of said protection.

Claims 30-32 have been added:

--30. The method of claim 13 wherein the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene, a C Erb  $\beta$ 2 gene or a TGF  $\alpha$  gene and wherein the cell type specific promoter is a human NF-L gene promoter.

31. The transgenic rat of claim 18 wherein the cell type specific promoter is a human NF-L gene promoter.

32. The method of claim 25 wherein the conditional oncogene, transforming gene or immortalizing gene or the cell cycle affecting gene is a SV40tsA58 gene, a C Erb  $\beta$ 2 gene or a TGF  $\alpha$  gene and wherein the cell type specific promoter is a human NF-L gene promoter.--